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Localization and analysis of engineered silver nanoparticles in *Pseudokirchneriella subcapitata*

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Silver nanoparticles have increased cytotoxic properties compared to larger particles, reflecting these properties, engineered silver nanoparticles are now added to an increasing number of consumer products often labelled as anti-bacterial. These particles are presently considered the fastest growing nanotechnology application. Accordingly, silver nanoparticles are now postulated to be released into the sewerage systems and wider environment in increasing quantities.

There are concerns that they could be harmful to aquatic organisms and disrupt the balance of bacteria (involved in the breakdown and processing of biological waste) in wastewater treatment facilities. Whether the size-related enhancement in silver nanoparticle toxicity is solely due to an increased release of silver ions or related to additional mechanisms for toxicity is still a matter of conjecture since there are studies supporting both theories. Furthermore, nanoparticles pose problems in experimental ecotoxicology model systems because they are highly heterogeneous in suspension and over time undergo processes such as aggregation, sedimentation, dissolution and changes in surface chemistry [1] – thus altering the dose. Recently a modified short-term model has been suggested, which could potentially increase the accuracy of algal growth inhibition tests with silver nanoparticles[2].

However, toxic mechanisms remain to be further elucidated and the uptake mechanism of these nanoparticles in aquatic organisms on an ultrastructural level play an important part of this.

Selenastrum capricornutum Printz (1913) CCAP 278/4 (*Pseudokirchneriella subcapitata* (Korschikov) Hindák 1990) is a microalgae which is routinely applied in eco toxicity tests.

In this study *P. subcapitata* were exposed to silver nanoparticles. They were then fixed with formaldehyde and glutaraldehyde, post fixed with osmium tetroxide, *en bloc* stained with uranyl acetate and dehydrated in graded series of ethanol. Finally they were embedded in Spurr's resin. The samples were either sectioned by ultramicrotomy and imaged by TEM, or they were imaged by serial block face sectioning in the FIB SEM or using the 3View system in SEM for localization of the silver nanoparticles within the organisms. Furthermore, EDS was employed to analyse the silver nanoparticles. The results of these different techniques will be presented and their implications discussed.

[1] NB Hartmann *et al*, Aquatic toxicology **118-119** (2012) p. 1.

[2] SN Sørensen and A Baun. Submitted to Nanotoxicology (2014).

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